Listing of the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

1. (Currently amended) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; ~~and~~

(b) contacting said cell with a serum-free, chemically defined cell culture medium comprising at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate; and

(c) cultivating said cell in suspension in said medium.

2. (Original) The method of claim 1, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

3. (Previously presented) The method of claim 2, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

4 - 5. (Cancelled)

6. (Original) The method of claim 1, wherein said medium is protein-free.

7. (Original) The method of claim 1, wherein said medium is a 1X medium formulation.

8. (Original) The method of claim 1, wherein said medium formulation is a 10X concentrated medium formulation.

9. (Previously presented) The method of claim 1, wherein said medium further comprises one or more ingredients selected from the group consisting of one or more amino acids, one or more vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, transferrin substitutes, insulin, and insulin substitutes.

10. (Previously presented) The method of claim 9, wherein said medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

11. (Original) The method of claim 10, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

12. (Original) The method of claim 9, wherein said amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

13. (Original) The method of claim 9, wherein said vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B_{12}.

14. (Original) The method of claim 9, wherein said inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, $Fe(NO_3)_3$, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts.

15. (Currently amended) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; ~~and~~

(b) contacting said cell with a serum-free, chemically defined cell culture medium comprising the ingredients ethanolamine, D-glucose, N-[2-

hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12}, at least one polyanionic or polycationic compound, one or more calcium salts, KCl, one or more iron salts, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

wherein each ingredient is present in an amount which supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate; and

(c) cultivating said cell in suspension in said medium.

16. (Original) The method of claim 15, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

17. (Previously presented) The method of claim 16, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

18 - 19. (Cancelled)

20. (Original) The method of claim 15, wherein said medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

21. (Original) The method of claim 20, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

22. (Currently amended) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; ~~and~~

(b) contacting said cell with a serum-free, chemically defined cell culture medium obtained by combining a basal medium with at least one polyanionic or polycationic compound, wherein said medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate; and

(c) cultivating said cell in suspension in said medium.

23. (Original) The method of claim 22, wherein said polyanionic compound is a polysulfonated or polysulfated compound.

24. (Previously presented) The method of claim 23, wherein said polysulfonated or polysulfated compound is selected from the group consisting of heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

25 - 26. (Cancelled)

27. (Original) The method of claim 22, wherein said basal medium is obtained by combining one or more ingredients selected from the group consisting of ethanolamine, D-glucose, N-[2-hydroxyethyl]-piperazine-N'-[2-ethanesulfonic acid] (HEPES), insulin, linoleic acid, lipoic acid, phenol red, PLURONIC F68, putrescine, sodium pyruvate, transferrin, L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, biotin, choline chloride, D-Ca^{++}-panthonate folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine, vitamin B_{12} one or more calcium salts, one or more iron salts, KCl, one or more magnesium salts, one or more manganese salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts,

wherein each ingredient is added in an amount which supports the cultivation of said cell in suspension.

28. (Original) The method of claim 22, wherein said medium is obtained by combining said basal medium and one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

29. (Original) The method of claim 28, wherein said one or more plant peptides are one or more rice peptides or one or more soy peptides.

30. (Original) The method of any one of claims 1, 15 or 22, wherein said mammalian cell is a mammalian epithelial cell.

31. (Original) The method of claim 30, wherein said mammalian epithelial cell is selected from the group consisting of a keratinocyte, a cervical epithelial cell, a bronchial epithelial cell, a tracheal epithelial cell, a kidney epithelial cell and a retinal epithelial cell.

32. (Original) The method of claim 30, wherein said cell is a human cell.

33. (Original) The method of claim 32, wherein said human cell is a 293 embryonic kidney cell, a HeLa cervical epithelial cell, a PER-C6 retinal cell, or a derivative thereof.

34. (Original) The method of claim 33, wherein said human cell is a 293 embryonic kidney cell.

35. (Original) The method of claim 30, wherein said cell is a normal cell.

36. (Original) The method of claim 30, wherein said cell is an abnormal cell.

37. (Original) The method of claim 36, wherein said abnormal cell is a transformed cell, an established cell, or a cell derived from a diseased tissue sample.

38 - 72. (Cancelled)

73. (Original) A method of producing a virus comprising

(a) obtaining a mammalian cell to be infected with a virus;

(b) contacting said cell with a virus under conditions suitable to promote the infection of said cell by said virus; and

(c) cultivating said cell according to the method of any one of claims 1, 15 or 22, under conditions suitable to promote the production of said virus by said cell.

74. (Original) The method of claim 73, wherein said mammalian cell is an epithelial cell.

75. (Original) The method of claim 73, wherein said mammalian cell is a human cell.

76. (Original) The method of claim 75, wherein said human cell is a 293 embryonic kidney cell.

77. (Original) The method of claim 73, wherein said virus is an adenovirus, an adeno-associated virus or a retrovirus.

78. (Cancelled)

79. (Withdrawn) A method of producing a polypeptide comprising

(a) obtaining a mammalian cell that has been genetically engineered to produce a polypeptide; and

(b) cultivating said cell according to the method of any one of claims 1, 15 or 22, under conditions favoring expression of said polypeptide by said mammalian cell.

80. (Withdrawn) The method of claim 79, wherein said mammalian cell is an epithelial cell.

81. (Withdrawn) The method of claim 79, wherein said mammalian cell is human cell.

82. (Withdrawn) The method of claim 81, wherein said human cell is a 293 embryonic kidney epithelial cell.

83 - 105. (Cancelled)

106. (Withdrawn) A method of cultivating mammalian cells in suspension culture to high density and/or expressing a recombinant protein, said method comprising the steps of

(a) contacting said cells with the eukaryotic cell culture medium of claim 84, wherein said Fe^{2+} chelate and said Zn^{2+} salt are each present in an amount which supports the growth of mammalian cells in culture; and

(b) cultivating said mammalian cells under conditions suitable to support the growth of said cells to high density and/or the expression of said recombinant protein.

107. (Withdrawn) The method according to claim 106, further comprising a polyanionic or polycationic compound, wherein said polyanionic or polycationic compound is present in an amount sufficient to prevent cell clumping and/or increase the level of recombinant protein expression.

108. (Withdrawn) The method according to claim 107, wherein said polyanionic compound is a polysulfonated compound or a polysulfated compound.

109. (Withdrawn) The method according to claim 108, wherein said polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

110 - 111. (Cancelled)

112. (Withdrawn) The method according to claims 106 or 107, wherein said eukaryotic cell culture medium further comprises one or more ingredients selected from the group consisting of L-arginine, L-asparagine-H_2O, L-aspartic acid, L-glutamic acid, L-histidine, hydroxy-L-proline, L-isoleucine, L-leucine, L-lysine·HCl, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine, L-valine, L-cysteine·2HCl, Na_2HPO_4, pyridoxine·HCl, thiamine·HCl, glutathione, cupric sulfate·$5H_2O$, cadmium chloride·$5H_2O$, cobalt chloride·$2H_2O$, stannous chloride·$2H_2O$, manganous sulfate·H_2O, nickel sulfate·$6H_2O$, sodium metavanadate, ammonium molybdate·$4H_2O$, barium acetate, potassium bromide, potassium iodide, chromium sulfate, sodium fluoride, silver nitrate, rubidium chloride, zirconyl chloride, aluminum chloride, germanium dioxide, titanium tetrachloride, sodium metasilicate, magnesium chloride (anhydrous), D-calcium pantothenate, calcium nitrate·$4H_2O$, potassium chloride, ascorbic acid magnesium salt phosphate, pluronic F68 10% solution, Na_2HPO_4, D-glucose, folic acid, riboflavin, biotin, choline chloride, niacinamide, i-inositol, sodium pyruvate, vitamin B-12, β-mercaptoethanol,

para-aminobenzoic acid, β-glycerophosphate, sodium selenite, ethanolamine·HCl, spermine, putrecine·2HCl, monothioglycerol, and sodium bicarbonate,

wherein each of said ingredients is present in an amount which supports the high-density growth of Chinese hamster ovary cells in suspension culture and/or the expression of recombinant protein.

113 - 139. (Cancelled)

140. (Previously presented) The method of claim 1, wherein said serum-free cell culture medium is free of animal-derived ingredients.

141 - 142. (Cancelled)

143. (Withdrawn) The method of claim 106, wherein said eukaryotic cell culture medium is free of animal-derived ingredients.

144. (Withdrawn) The method of claim 106, wherein said eukaryotic cell culture medium is protein-free.

145. (Withdrawn) The method of claim 106, wherein said eukaryotic cell culture medium is chemically defined.

146. (Withdrawn) The method of claim 106, wherein said eukaryotic cell culture medium contains neither transferrin nor insulin.

147. (Withdrawn) The method of claim 106, wherein said mammalian cells are Chinese hamster ovary cells.

148. (Withdrawn) The method of claim 106, wherein said eukaryotic cell culture medium is a 1X medium formulation.

149. (Withdrawn) The method of claim 106, wherein said eukaryotic cell culture medium is a concentrated medium formulation.

150. (Withdrawn) The method of claim 149, wherein said eukaryotic cell culture medium is a 10X medium formulation.

151. (Withdrawn) The method of claim 149, wherein said eukaryotic cell culture medium formulation is greater than 10X.

152. (Withdrawn) The method of claim 106, wherein the concentration of said Fe^{2+} is about 0.00028 to 0.011 g/L, and said concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

153. (Withdrawn) The method of claim 152, wherein said concentration of said Fe^{2+} is about 0.0011 g/L, and said concentration of Zn^{2+} is about 0.000354 g/L.

154. (Cancelled)

155. (Withdrawn) The method of claim 106, wherein said medium is serum-free, and wherein said growth is high-density growth.

156. (Withdrawn) The method of claim 112, wherein said growth is high-density growth.

157. (Previously presented) A method for replacing protein in a mammalian cell culture medium, said method comprising

replacing insulin with a Zn^{2+} salt and replacing transferrin with a Fe^{2+} chelate and/or a Fe^{3+} chelate.

158. (Currently amended) A method of cultivating a mammalian cell in suspension *in vitro*, comprising:

(a) obtaining a mammalian cell to be cultivated in suspension; ~~and~~

(b) contacting said cell with a serum-free, non-animal derived cell culture medium comprising at least one polyanionic or polycationic compound, wherein said

medium supports the cultivation of said cell in suspension, with the proviso that the medium does not contain dextran sulfate; and

(c) cultivating said cell in suspension in said medium.

159. (Previously presented) The method of claim 157, wherein Fe^{2+} and/or Fe^{3+} is present at a concentration of about 0.00028 to 0.011 g/L, and the concentration of Zn^{2+} is about 0.00007 to 0.00073 g/L.

160. (Previously presented) The method of claim 159, wherein the concentration of Fe^{2+} and/or Fe^{3+} is about 0.0011 g/L and the concentration of Zn^{2+} is about 0.000354 g/L.

161. (Currently amended) A method of cultivating 293 cells in suspension *in vitro*, comprising:

(a) obtaining 293 cells to be cultivated in suspension; ~~and~~

(b) contacting the cells with a serum-free, chemically defined cell culture medium, wherein the medium supports the cultivation of the cell in suspension; and

(c) cultivating said cells in suspension in said medium.

162. (Previously presented) The method of claim 161, wherein the medium further comprises at least one polyanionic or polycationic compound.

163. (Previously presented) The method of claim 162, wherein the polyanionic compound is a polysulfonated compound or a polysulfated compound.

164. (Previously presented) The method of claim 163, wherein the polysulfonated or polysulfated compound is selected from the group consisting of dextran sulfate, heparin, heparan sulfate, chondroitin sulfate, dermatan sulfate, pentosan sulfate and a proteoglycan.

165. (Previously presented) The method of claim 162, wherein the polysulfonated or polysulfated compound is dextran sulfate.

166. (Previously presented) The method of claim 165, wherein the dextran sulfate has an average molecular weight of about 5,000 dalton.

167. (Previously presented) The method of claim 162, wherein the medium is protein-free.

168. (Previously presented) The method of claim 162, wherein the medium further comprises one or more ingredients selected from the group consisting of one or more amino acids, one or more vitamins, one or more inorganic salts, one or more buffering salts, one or more sugars, one or more lipids, transferrin, one or more transferrin substitutes, insulin, and one or more insulin substitutes.

169. (Previously presented) The method of claim 168, wherein the medium further comprises one or more supplements selected from the group consisting of one or more cytokines, heparin, one or more animal peptides, one or more yeast peptides and one or more plant peptides.

170. (Previously presented) The method of claim 169, wherein the one or more plant peptides are one or more rice peptides or one or more soy peptides.

171. (Previously presented) The method of claim 168, wherein the amino acid ingredient comprises one or more amino acids selected from the group consisting of L-alanine, L-arginine, L-asparagine, L-aspartic acid, L-cysteine, L-glutamic acid, L-glutamine, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine.

172. (Previously presented) The method of claim 168, wherein the vitamin ingredient comprises one or more vitamins selected from the group consisting of biotin, choline chloride, D-Ca^{++}-pantothenate, folic acid, *i*-inositol, niacinamide, pyridoxine, riboflavin, thiamine and vitamin B_{12}.

173. (Previously presented) The method of claim 168, wherein the inorganic salt ingredient comprises one or more inorganic salts selected from the group consisting of one or more calcium salts, $Fe(NO_3)_3$, KCl, one or more magnesium salts, one or more manganese

salts, NaCl, $NaHCO_3$, Na_2HPO_4, one or more selenium salts, one or more vanadium salts and one or more zinc salts.

174. (Previously presented) The method of claim 165, wherein the dextran sulfate is present in the medium in an amount effective to substantially prevent clumping.